Report for 2004KY41B: Linking chemical tolerance to reproductive resilience: CYP1A as a metric for predicting fish species distributions in chemically impacted habitats

- Articles in Refereed Scientific Journals:
  - Brammell, Benjamin F., David J. Price, Wesley J. Birge, and Adria A. Eslkus, 2004, Apparent Lack of CYP1A1 Response to High PCB Body Burdens in Fish from a Chronically Contaminated PCB Site, Marine Environmental Research, 58(2004), p. 251-255.
- Conference Proceedings:
  - Brammell, Benjamin F., Eleana M. Harmel, John A. Hitron, Xabier Arzuaga, David J. Price, Wesley J. Birge, and Adria A. Elskus, 2005, Induction of Pollutant Metabolozing Enzymes in Lepomis Species Following PCB and PAH Exposure, in Proccedings Kentucky Water Resources Annual Symposium, March 3, 2005, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 85.
  - Brammell, Benjamin F., Eleana M. Harmel, J. Andrew Hitron, Xabier Arzuaga, David J. Price, Wesley J. Birge, and Adria Elskus, 2004, Induction of Pollutant Metabolizing Enzymes in Lepomis Species Following PCB and PAH Exposure, in Proceedings Society of Environmental Toxicology and Chemistry, 25th Annual Meeting, November 14-18, 2004, Portland Oregon, SETAC, p. 269.

Report Follows

Problem and Research Objectives

Polychlorinated biphenyls (PCBs) are ubiquitous aquatic pollutants with significant toxic effects in both humans and fish. In our previous KWRRI-funded projects (2002KY1B, 2003KY17B), we found both sediments and fish in the Town Branch-Mud River (TB/MR) system in Kentucky to be highly contaminated with PCBs, despite extensive remediation efforts by the state and others to remove these chemicals. We also found resident fish in the TB/MR system had developed resistance to some of the toxic effects of these chemicals, including induction of the biomarker enzyme, CYP1A. Based on research in our laboratory and others, we **hypothesized** that there is a mechanistic link between resistance to PCB mediated induction of CYP1A and resistance to the deleterious effects of PCBs on reproductive function.

Original objectives: 1) Determine if PCB-resistant resident populations in Town Branch have altered reproductive function compared to non-resistant populations of the same and different species; 2) Determine whether species abundance reflects species-specific ability to develop resistance; 3) Determine whether altered regulation of the pollutant-metabolizing enzyme, CYP1A (a defining characteristic of PCB-resistance in fish) is mechanistically linked to reproductive function.

Methodology

We were unable to pursue two of our original objectives due to unexpected remediation of our field site which resulted in a massive disturbance of the entire Town Branch study area. The remediation occurred in April 2004 and involved removal of large amounts of sediment from the bottom and banks of Town Branch stream leading to a virtual absence of fish in the study area. This development forced us to abandon objective 2 (a field survey of resident species) and modify objective 1. Objective 3 remained, but we had to switch to a non-resident fish species to address it.

Modified objective # 1: Determine whether acquired resistance to PCBs, as measured by altered expression of CYP1A, results in resistance to the endocrine disrupting effects of PCBs.

Modified Methodology

To meet the new objective 1, we modified our original method, evaluating endocrine function in PCB-resistant and responsive resident TB/MR fish, to use PCB-resistant and PCB-responsive populations of the killifish (*Fundulus heteroclitus*). For the killifish study, we chose thyroid function, instead of reproductive function, as the endocrine endpoint. Briefly, killifish were depurated, treated with PCBs or vehicle, and evaluated for CYP1A activity, circulating levels of the thyroid hormone T3, T4 and activity of UDP-GT, an enzyme involved in thyroid hormone metabolism.

Original objective #3: To determine whether altered regulation of the pollutant-metabolizing enzyme, CYP1A (a defining characteristic of PCB-resistance in fish) is mechanistically linked to reproductive function.

Modified Methodology
To meet objective 3, we used rainbow trout, instead of TB/MR resident species, as the source of hepatocytes to evaluate PCB effects on reproductive function. As originally proposed, we used green sunfish as the PCB-resistant species. No other major changes in methodology were made.

Principal Findings and Significance

In fish, resistance to PCBs is characterized, in large part, by the ability of PCBs to induce the biomarker enzyme, CYP1A. We asked whether resistance to PCB-induction of CYP1A indicates resistance to the toxic effects of PCBs, specifically, their endocrine-disrupting effects. If this proved to be the case, CYP1A might be used as an indicator of response to chemicals that affect both CYP1A and endocrine function.

Our results suggest that resistance to PCB induction of the biomarker, CYP1A, may reflect resistance to the endocrine-disrupting effects of PCBs. Our *in vivo* studies with resistant and responsive killifish suggest that fish which are resistant to induction of CYP1A by PCBs may also be resistant to the deleterious effects of PCBs on thyroid hormone metabolism, based on UDP-GT and T4 levels (T3 assays are currently underway). Because inter-individual variability was higher than expected, the thyroid hormone data must be considered preliminary, indicating trends, not statistical differences.

Preliminary data from our *in vitro* studies with rainbow trout and green sunfish hepatocytes confirm our earlier *in vivo* studies that green sunfish, as a species, are resistant to PCB induction of CYP1A while rainbow trout are responsive. Completion of the egg-yolk protein analysis from these *in vitro* studies (expected June 2005) will determine if lack of CYP1A response to PCBs protects fish from reproductive disruption by PCBs, specifically, suppression of egg-yolk protein production by PCB treatment.